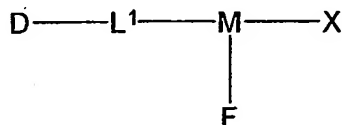


Claims

1. A compound selected from an acridone and a quinacridone dye
containing at least one target bonding group selected from a carboxylic acid
5 thioester group or a group suitable for covalent reaction with a thioester,
wherein said compound optionally includes an affinity tag covalently bound
thereto.

2. A compound according to claim 1 having the formula (I):

10



(I)

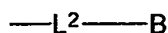
15 wherein:

D is a fluorescent dye selected from an acridone and a quinacridone dye;

F comprises a target bonding group selected from a carboxylic acid thioester
group and a 1,2-aminothiol group;

M is a group adapted for attaching to F;

20 X is selected from hydrogen or the group:

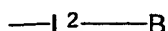


wherein B is an affinity tag; and

25 L¹ and L² each independently comprise a group containing from 1 – 40 linked
atoms selected from carbon atoms which may optionally include one or more
groups selected from –NR'–, –O–, –CH=CH–, –CO–NH– and phenylenyl
groups, where R' is selected from hydrogen and C₁ – C₄ alkyl.

3. A compound according to claim 2 wherein X is the group:

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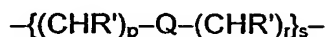


wherein B and L² are hereinbefore defined.

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4. A compound according to claim 2 or claim 3 wherein each of L¹ and L² contains from 2 to 30 atoms.

5. A compound according to claim 2 or claim 3 wherein L¹ and L² are
5 independently selected from the group:



where Q is selected from: -CHR'-, -NR'-, -O-, -CH=CH-, -Ar- and
10 -CO-NH-; R' is hydrogen or C₁ - C₄ alkyl, p is 0 - 5, r is 1 - 5 and s is 1 or 2.

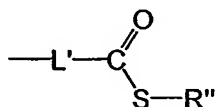
6. A compound according to claim 5 wherein Q is selected from -CHR'-, -O- and -CO-NH-, where R' is hereinbefore defined.

15 7. A compound according to any of claims 1 to 6 wherein said affinity tag is selected from biotin and desthiobiotin.

8. A compound according to any of claims 1 to 6 wherein said affinity tag is selected from his-tag, iminodiacetic acid and nitrilotriacetic acid.

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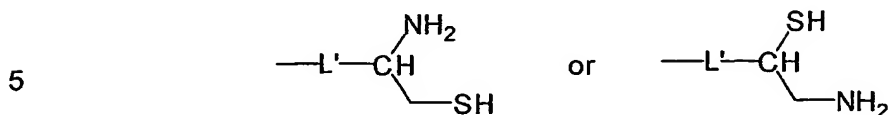
9. A compound according to any of claims 1 to 8 wherein the target bonding group F is a carboxylic acid thioester of formula:



25

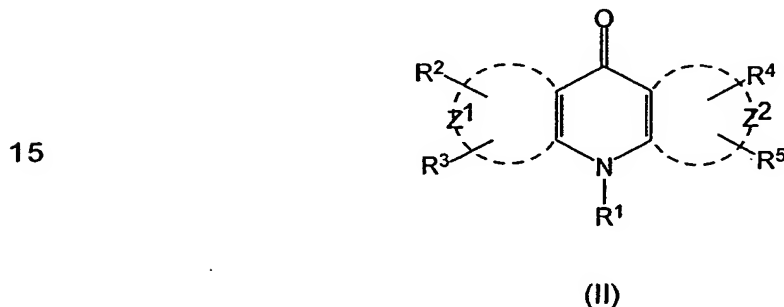
wherein L' is a bond or is a group containing from 1 - 30 linked atoms selected from carbon atoms and optionally one or more groups selected from -NH-, -O- and -CO-NH-; and R'' is C₁ - C₄ alkyl, C₆ - C₁₀ aryl, or C₇ - C₁₅
30 aralkyl, which may be optionally substituted with sulphonate; or is the group -(CH₂)₂-CONH₂.

10. A compound according to any of claims 1 to 8 wherein the target bonding group F is a 1,2-aminothiol group of formula:



wherein L' is hereinbefore defined.

11. A compound according to any of claims 1 to 10 wherein the compound is an acridone dye having the formula (II):



wherein:

- 20 groups R² and R³ are attached to the Z¹ ring structure and groups R⁴ and R⁵ are attached to the Z² ring structure;
 Z¹ and Z² independently represent the atoms necessary to complete one ring or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected from carbon atoms and optionally no more than two atoms
 25 selected from oxygen, nitrogen and sulphur;
 at least one of groups R¹, R², R³, R⁴ and R⁵ is a group W having the formula:

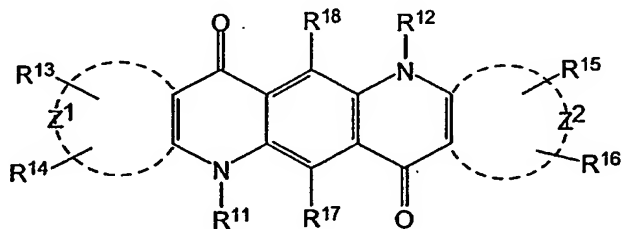


where F, M, X and L¹ are hereinbefore defined;

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when any of said groups R^1 , R^2 , R^3 , R^4 and R^5 is not said group W, said remaining groups R^2 , R^3 , R^4 and R^5 are independently selected from hydrogen, halogen, amide, cyano, mono- or di- $C_1 - C_4$ alkyl-substituted amino, carbonyl, carboxyl, $C_1 - C_6$ alkoxy, acrylate, vinyl, styryl, aryl, heteroaryl, $C_1 - C_{20}$ alkyl, aralkyl, sulphonate, sulphonic acid, quaternary ammonium and the group $-(CH_2)_n-Y$ and, when group R^1 is not said group W, it is selected from hydrogen, $C_1 - C_{20}$ alkyl, aralkyl and the group $-(CH_2)_n-Y$; and Y is selected from sulphonate, sulphate, phosphonate, phosphate, quaternary ammonium and carboxyl; and n is an integer from 1 to 6; provided that at least one of groups R^1 , R^2 , R^3 , R^4 and R^5 is a water solubilising group.

12. A compound according to any of claims 1 to 10 wherein the compound is a quinacridone dye having the formula (III):



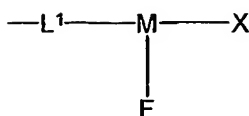
(III)

wherein:

groups R^{13} and R^{14} are attached to the Z^1 ring structure and groups R^{15} and R^{16} are attached to the Z^2 ring structure; Z^1 and Z^2 independently represent the atoms necessary to complete one ring or two fused ring aromatic or heteroaromatic systems, each ring having five or six atoms selected from carbon atoms and optionally no more than two atoms selected from oxygen, nitrogen and sulphur; at least one of groups R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} and R^{18} is a group T having the formula:

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- 5 where F, M, X and L¹ are hereinbefore defined;
 when any of said groups R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷ and R¹⁸ is not said group T,
 said remaining groups R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷ and R¹⁸ are independently
 selected from hydrogen, halogen, amide, cyano, mono- or di-C₁ – C₄ alkyl-
 substituted amino, carbonyl, carboxyl, C₁ – C₆ alkoxy, acrylate, vinyl, styryl,
 10 aryl, heteroaryl, C₁ – C₂₀ alkyl, aralkyl, sulphonate, sulphonic acid, quaternary
 ammonium and the group –(CH₂)_n–Y; and,
 when either of groups R¹¹ and R¹² is not said group T, it is selected from
 hydrogen, C₁ – C₂₀ alkyl, aralkyl and the group –(CH₂)_n–Y;
 Y is selected from sulphonate, sulphate, phosphonate, phosphate, quaternary
 15 ammonium and carboxyl; and n is an integer from 1 to 6;
 provided that at least one of groups R¹¹, R¹², R¹³, R¹⁴, R¹⁵, R¹⁶, R¹⁷ and R¹⁸ is
 a water solubilising group.

13. A compound according to claim 11 or claim 12 wherein Z¹ and Z² are
 20 selected independently from the group consisting of phenyl, pyridinyl,
 naphthyl, quinolinyl and indolyl moieties.

14. A compound according to claim 11 or claim 12 wherein Z¹ and Z² are
 selected from phenyl and naphthyl moieties.

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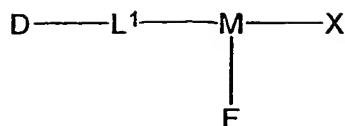
15. A method for labelling a protein of interest wherein said protein
 contains or is derivatised to contain an N-terminal cysteine, the method
 comprising:

- i) adding to a liquid containing said protein a compound of formula (I):

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(I)

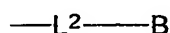
wherein:

D is a fluorescent dye selected from an acridone and a quinacridone dye;

F comprises a target bonding group selected from a carboxylic acid thioester group and a 1,2-aminothiol group;

10 M is a group adapted for attaching to F;

X is selected from hydrogen or the group:



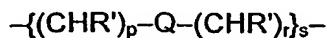
where B is an affinity tag; and

15 L¹ and L² each independently comprise a group containing from 1 – 40 linked atoms selected from carbon atoms which may optionally include one or more groups selected from –NR'–, –O–, –CH=CH–, –CO–NH– and phenylenyl groups, where R' is selected from hydrogen and C₁ – C₄ alkyl; and

20 ii) incubating said compound with said protein under conditions suitable for labelling said protein.

16. A compound according to claim 15 wherein each of L¹ and L² contains from 2 to 30 atoms.

25 17. A method according to claim 15 wherein L¹ and L² are independently selected from the group:



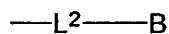
30 where Q is selected from: –CHR'–, –NR'–, –O–, –CH=CH–, –Ar– and –CO–NH–; R' is hydrogen or C₁ – C₄ alkyl, p is 0 – 5, r is 1 – 5 and s is 1 or 2.

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18. A method according to claim 17 wherein Q is selected from -CHR'-, -O- and -CO-NH-, where R' is hereinbefore defined.

19. A method according to any of claims 15 to 18 wherein X is the group:

5



wherein B and L² are hereinbefore defined, said method further comprising separating and/or purifying the dye-labelled protein of interest by affinity chromatography.

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20. A method according to any of claims 15 to 19 wherein said protein of interest is selected from antibody, antigen, protein, peptide, microbial materials, cells and cell membranes.

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